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Source: Malacologia, 58(1/2):129-145.

Published By: Institute of Malacology

DOI: <http://dx.doi.org/10.4002/040.058.0202>

URL: <http://www.bioone.org/doi/full/10.4002/040.058.0202>

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## GROWTH AND BODY WEIGHT VARIABILITY OF THE INVASIVE MUSSEL *LIMNOPERNA FORTUNEI* (MYTILIDAE) ACROSS HABITAT AND SEASON

Nicolás Bonel<sup>1,2\*</sup> & Julio Lorda<sup>3,4</sup>

### ABSTRACT

The freshwater mussel *Limnoperna fortunei* is adapted to colonize a wide range of aquatic environments, and its ability to contend with environmental stress through phenotypic plasticity has allowed this species to successfully colonize and become established in new regions. Only limited information is currently available on the wide intraspecific variability of this species in response to environmental heterogeneity. Here, we tested the hypotheses that (1) growth and body weight of mussels from a highly polluted environment differed from those from a less polluted habitat, and (2) growth parameters estimated in this study differed from those reported for other invaded ecosystems. We conducted controlled field experiments in two study sites with differing levels of pollution. To compare our results to those reported elsewhere, we considered growth data from studies performed in different locations. We found that mussels from the more polluted habitat showed lower shell growth and body weight than those from the less polluted environment. We also observed differences in the growth performances of the golden mussel between our estimates and those from other invaded habitats. Our findings provide useful information to better understand the striking intraspecific variability of this species in response to stressful conditions. Knowledge on the phenotypic plasticity of *L. fortunei* is essential for predicting and managing this species.

Key words: invasive species, freshwater bivalve, golden mussel, population dynamics, growth model, weight, water pollution.

### INTRODUCTION

Understanding the temporal and spatial variability of invasive species' populations is essential for accurate predictive power and effective management of these populations. Traits common to invasive species include *r*-selected life histories, such as use of pioneer habit, short generation time, high fecundity and high growth rates. In addition, through phenotypic plasticity – adaptation to environmental stress – and high tolerance to environmental heterogeneity, non-native species are able to colonize and successfully establish in new areas (Sakai et al., 2001).

One successful invader, the freshwater golden mussel, *Limnoperna fortunei* (Dunker, 1857) is a bivalve native to Southeast Asia that has been introduced into other regions of Asia and South America over the last few decades (Pastorino et

al., 1993). In general, the available information on *L. fortunei* includes its geographic distribution, ecologic interactions, reproductive activity, ecological and economic impacts, and some aspects of its population dynamics (summarized in Boltovskoy et al., 2009). However, most of the biological and ecological studies of *L. fortunei* have been restricted to specific locations, and therefore the performance of *L. fortunei* among different habitats is unknown (Boltovskoy et al., 2009). Recently, Bonel et al. (2013) found spatial differences in density and growth. They observed that these biological parameters were lower in a more polluted environment, but the growth data obtained from settlement plates in that study was better described by a linear model and did not account for the effect of seasonality on growth.

The life history of an organism implies the allocation of its resources among maintenance,

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growth, and reproduction, which changes over time with age and/or season (Harvey & Vincent, 1990; Chase & Bailey, 1999) and across geography within different habitats. The growth of *L. fortunei* has been independently estimated for populations established in different habitats in the Neotropical Region and in Asia (Boltovskoy & Cataldo, 1999; Maroñas et al., 2003; dos Santos et al., 2008; Belz et al., 2010; Darrigran et al., 2011; Nakano et al., 2011; Bonel et al., 2013). In bivalves, the magnitude of weight variability over season has been used to estimate fecundity, allocation of resources to reproduction (Hilbish, 1986), and as a general index of physiological condition (Nalepa et al., 1995). Sylvester et al. (2007) estimated the weight-to-length relationship for individuals of *L. fortunei* collected from a single sampling point in the Parana River in South America. However, studies analyzing the spatial and temporal changes in body weight are still inchoate or nil for the golden mussel.

In this paper we address the question of how individual growth and body weight of *L. fortunei* varies in response to environmental heterogeneity and season. Based on data obtained from controlled field experiments in two study sites with differing levels of pollution, we ask whether the high levels of water pollution decrease growth and body weight of *L. fortunei* despite its high tolerance to environmental stress. We also tested the hypothesis that the growth parameters estimated here differed from the corresponding data for mussels established in other habitats worldwide as evidence of the high phenotypic plasticity of *L. fortunei* to environmental heterogeneity. Except for Nakano et al. (2011), the sites referred in those studies we compared do not provide environmental information (Boltovskoy & Cataldo, 1999; Maroñas et al., 2003; dos Santos et al., 2008; Belz et al., 2010; Darrigran et al., 2011). We therefore assume that they are environmentally different from our study sites because they belong to different river systems at different latitudes. Comparative studies are essential in the field of invasion ecology because non-indigenous species' populations and their impacts are not homogeneous in space or time (Boltovskoy et al., 2009). Within the context of global change, the comparative approach of this study will be of great value for understanding the ecology and evolutionary biology of the golden mussel.

## METHODS

### Study Sites

To perform this study, we selected two water bodies from the Río de la Plata basin, Argentina. The first site was in the Coronda River, a secondary course of the Paraná River (31°41'26.88"S, 60°44'34.08"W). The second site was the Santiago River (34°51'5.76"S, 57°53'29.76"W) located 350 km southeast of the first sampling point. Environmental conditions for the two river systems are fully described in a previous study where the Santiago River is defined as the more polluted environment of the two sites (Bonel et al., 2013).

### Sampling Procedure

To study the individual growth and body weight of *Limnoperna fortunei*, we conducted controlled field experiments by following cohorts of mussels placed in cages deployed at the Santiago River and the Coronda River starting in March 2007. The cages had tetrahedral shape (edge: 45 cm, height: 42 cm, basis: 30 cm) in order to diminish resistance to water current. The internal volume of each cage was approximately 12,700 cm<sup>3</sup>. Each cage was lined with 1 mm plastic mesh screen to prevent small mussels from escaping and to avoid predation from crabs and fish while allowing water circulation through the cage. However, the cages also allowed entrance of *L. fortunei* larvae. The efficiency of the cage design was tested in a previous study (Darrigran et al., 2011).

First, we collected juveniles of *L. fortunei* from both study sites. Then, we measured the total length (*L*; maximum anteroposterior axis) of all individuals to the nearest 0.01 mm with digital Mitutoyo calipers. In the Coronda River we deployed two cages with 397 and 427 individuals per cage. The average ( $\pm$  SD) shell-length within each cage was 4.27  $\pm$  0.82 and 4.12  $\pm$  0.84 mm; respectively. In the Santiago River we placed three cages with 457, 410, and 452 juveniles per cage with an average ( $\pm$  SD) shell-length of 4.41  $\pm$  0.63, 4.23  $\pm$  0.57, and 4.14  $\pm$  0.67 mm; respectively. Cages were suspended one meter in depth in the rivers and were spaced ten meters apart within the same river. We considered all experimental mussels to be within the same cohort, given their simi-

larity in shell-length (Lévêque, 1971; Vakily, 1992). Mussels attached readily to the interior of the cages, including the plastic mesh, and were usually found in clumps.

Monthly, we randomly subsampled mussels without replacement in both rivers. In the Coronda River we collected an average ( $\pm$  SD) of  $21 \pm 5$  individuals per cage from April 2007 through October 2007. However, when recruitment was observed in December 2007, the average number of individuals subsampled from that month through December 2008 was  $184 \pm 100$ . In the Santiago River, we collected  $23 \pm 5$  individuals from May 2007 through September 2007 prior to recruitment, and  $337 \pm 260$  from November 2007 through April 2009 after recruitment.

#### Size-Structure, Cohort Analysis, and Shell Morphometric Ratios

We measured the shell-length of all individuals collected from each cage. The size-class interval for length-frequency distributions was calculated by means of the Sturges's method previously described by Bonel et al. (2013). To identify cohorts by means of the length-frequency distributions we pooled shell-length data from each replicate and applied the Bhattacharya's method available in FISAT II software (Version 1.2.0, FAO-ICLARM Fish Assessment Tools; Gayanilo et al., 2002). To confirm each component normal distributions from the modal progression analysis, we used the NORMSEP method also available in the FISAT II software (Pauly & Caddy, 1985).

As noted above the mesh screen of cages allowed water circulation as well as entrance of larvae of *L. fortunei*. Bonel et al. (2013) observed that high mussel density yields individuals with more elongated shells, that is, higher length-to-width ratio. Changes in shell morphometry of enclosed mussels could occur due to possible effects of high density or environmental heterogeneity. Thus, it would be likely to find elongated or flattened – length-to-width ratio – mussels in more densely populated cages. Therefore, in those individuals with shell length larger than 3 mm, we additionally measured height and width to compare morphometric ratios between the linear shell dimensions length-to-width (L/W), length-to-height (L/H), and height-to-width (H/W). We transformed the ratios to arc-sine square roots to meet normality and homoscedastic condi-

tions, and we used two-tail *t*-tests to make comparisons between the groups.

#### Individual Growth

Growth curves were represented by a modified von Bertalanffy growth function (VBGF) with seasonal oscillations, according to Pauly & Gaschütz (1979), Hoenig & Choudary Hanumara (1982), and Somers (1988):

$$L_t = L_\infty (1 - \exp^{-K[(t-t_0) + T_1 - T_2]})$$

$$T_1 = C \sin(2\pi(t - t_s) / 2\pi)$$

$$T_2 = C \sin(2\pi(t_0 - t_s) / 2\pi)$$

where  $L_t$  is the predicted length at age  $t$ ;  $L_\infty$  is the asymptotic length,  $K$  is the growth constant of dimension time<sup>-1</sup> (year<sup>-1</sup> in most seasonally oscillating growth curves) expressing the rate at which  $L_\infty$  is approached;  $t_0$  is the theoretical 'age' the mussel have at length zero;  $C$  expresses the relative amplitude of the seasonal oscillation and varies between 0 and 1 (0 indicating lack of summer-winter differences in growth); and  $t_s$  is the starting point of the oscillation. For visualization, we define the period when growth is slowest as a fraction of the year called the Winter Point (WP =  $t_s + 0.5$ ). The parameters of the function were estimated by the modeling method available in JMP statistical software (v9.0 SAS Institute) which uses the iterative non-linear Gauss-Newton algorithm. Preliminary results of the oscillating VBGF failed to estimate the asymptotic shell-length ( $L_\infty$ ). Therefore, we used the maximum shell-length ( $L_{max}$ ) observed in caged conditions at the Coronda and Santiago rivers ( $L_{max} = 25.22$  and  $26.17$  mm, respectively) to calculate the asymptotic shell-length following the equation suggested by Taylor (1958):

$$L_{max} / 0.95 = L_\infty$$

The asymptotic shell-length obtained for the Coronda River was  $L_\infty = 26.55$  mm while for the Santiago mussels was  $L_\infty = 27.55$  mm. These values were fixed when we performed a second fit. We used the sum of the squares from the *F*-test to compare growth models fitted to cohorts with similar  $t_0$  from each study site. A non-significant *F* statistic indicates that a single growth curve is sufficient to describe the populations being compared (Blanchard & Feder, 2000; Bonel et al., 2013). As  $t_0$  is the theoretical 'age' the mussel have at length zero, we could define  $t_{max}$  as the theoretical 'age' the mussel reaches its maximum length ( $L_{max}$ ). Since  $L_{max}$  is dependently related to a maximum point in time (years), we therefore assume that  $L_{max}$

implicates  $t_{max}$  ( $L_{max} \Rightarrow t_{max}$ ). Thus, we could approximately express theoretical longevity of a mussel cohort (in years) as:

$$\text{longevity} = t_{max} - t_0$$

We calculated the daily growth rate (GR) for each mussel by dividing the increase in length during each growth interval by the number of days elapsed (Borrero & Hilbish, 1988). To analyze and compare temporal variation of growth rates between sites, we performed a two-way ANOVA and post-hoc Tukey HSD to test for differences among seasons and sites.

To compare growth in terms of length, we used the growth-performance index, phi prime ( $\phi'$ ) defined by Pauly & Munro (1984) as:

$$\phi' = 2 \log_{10} L_{\infty} + \log_{10} K$$

This criterion was chosen because the negative correlation between growth parameters ( $K$  and  $L_{\infty}$ ) invalidated comparisons based on individual parameters (Vakily, 1992; Ramón et al., 2007). We calculated the growth-performance index using the Growth Performance Indices application available in FISAT II software (Gayanilo et al., 2002), and we performed a  $t$ -test to compare the indices between the two populations studied here. Alternatively, in order to compare our results to those obtained in other studies we had to calculate growth-performance indices ( $\phi'$ ) from growth parameters provided in ecological studies of *L. fortunei* that used the von Bertalanffy growth function (Boltovskoy & Cataldo, 1999; Maroñas et al., 2003; Belz et al., 2010). By means of one-sample  $t$ -test, we tested whether the average growth-performance indices estimated here, and for each study site, differed significantly from those calculated from  $K$  and  $L_{\infty}$  reported in published studies.

### Body Weight

To analyze and compare body weight between individuals from each site, we used a subsample of individuals collected from cages from the Coronda River ( $n = 706$ ) and the Santiago River ( $n = 1,275$ ) following the length-frequency distribution obtained previously for each date and site.

Previous experiments indicated that the weight of individuals lower than 5 mm shell-length was too small for accurate weight measurements, so they were excluded from the analysis. With a scouring pad, we removed byssal threads, sediment, and epibiota before

weighing and measuring the organic content of the mussel. We dried individual mussels in porcelain crucibles for 48 hours at 60°C, weighed the mussels with a digital scale (precision, 0.1 mg), ashed the mussels for two hours in a muffle furnace at 500°C, and then reweighed them (Nalepa et al., 1995). Weight values were grouped into size class intervals constructed from length measurements of subsampled mussels.

We analyzed the relationship between ash-free dry weight (AFDW) and length with the allometric equation:

$$\text{AFDW} = a\text{Length}^b$$

where  $a$  is the intercept and  $b$  the allometric coefficient (or slope) of the equation. The allometric regressions were calculated separately for the two populations from the Coronda and Santiago rivers. The parameters of the allometric function were estimated by the modeling method in JMP statistical software (v9.0 SAS Institute) previously described. The correspondence of the allometric curves fitted to different groups was then tested through the use of the sum of the squares from the  $F$ -test explained in the *individual growth* methods section. To analyze the effect of spatial variation, we performed an ANCOVA with AFDW as the dependent variable, length as a covariate, and sites as factors. Shell-length and AFDW data were natural-log transformed to obtain linear relationship and to meet the assumptions of normality and homoscedasticity.

Alternatively, we defined the corresponding season (i.e., summer, autumn, winter, and spring) based on the sampling date, and we compared seasonal variation of the average AFDW between sites using two-way ANOVA and post-hoc Tukey HSD to test for differences among seasons and sites. To analyze the relationship between daily growth rates and AFDW of each cohort from each study site, we used nonparametric Spearman's rank correlation test.

## RESULTS

### Size-Structure, Cohort Analysis, and Shell Morphometric Ratios

We constructed length-frequency distributions based on the length values of 4,870 mussels from the Coronda River from March 2007 through December 2008 (Fig. 1). Polymodal

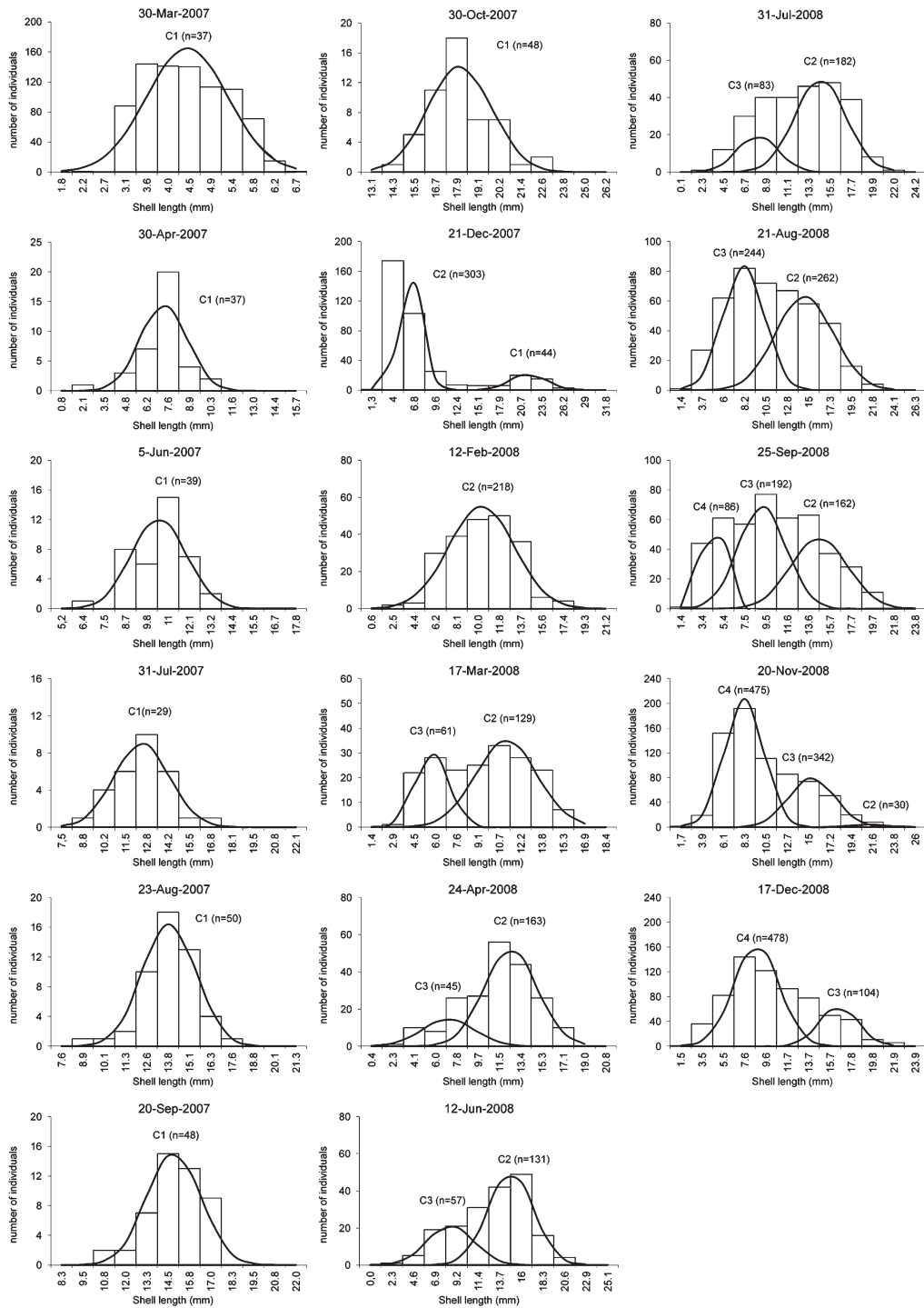


FIG. 1. Length-frequency distribution of *Limnoperna fortunei* from the Coronda River from March 2007 through December 2008; C, cohort; n, number of mussels from each cohort.

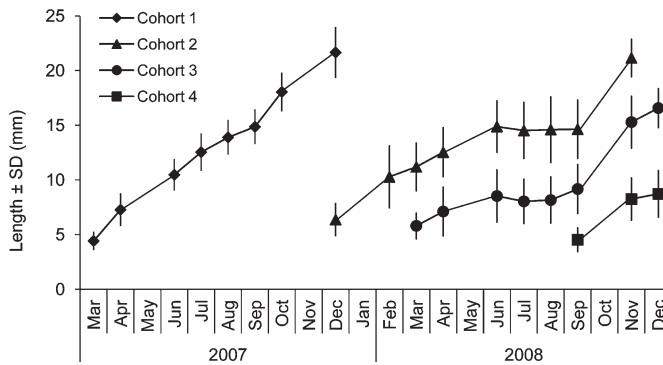


FIG. 2. Cohorts identified from length-frequency distributions. *SD*, standard deviation.

decomposition identified four cohorts throughout the study period (Fig. 2). In the Santiago River, we constructed the length-frequency distributions using 13,582 mussels sampled from March 2007 through April 2009 (Fig. 3) and we identified five cohorts (Fig. 4).

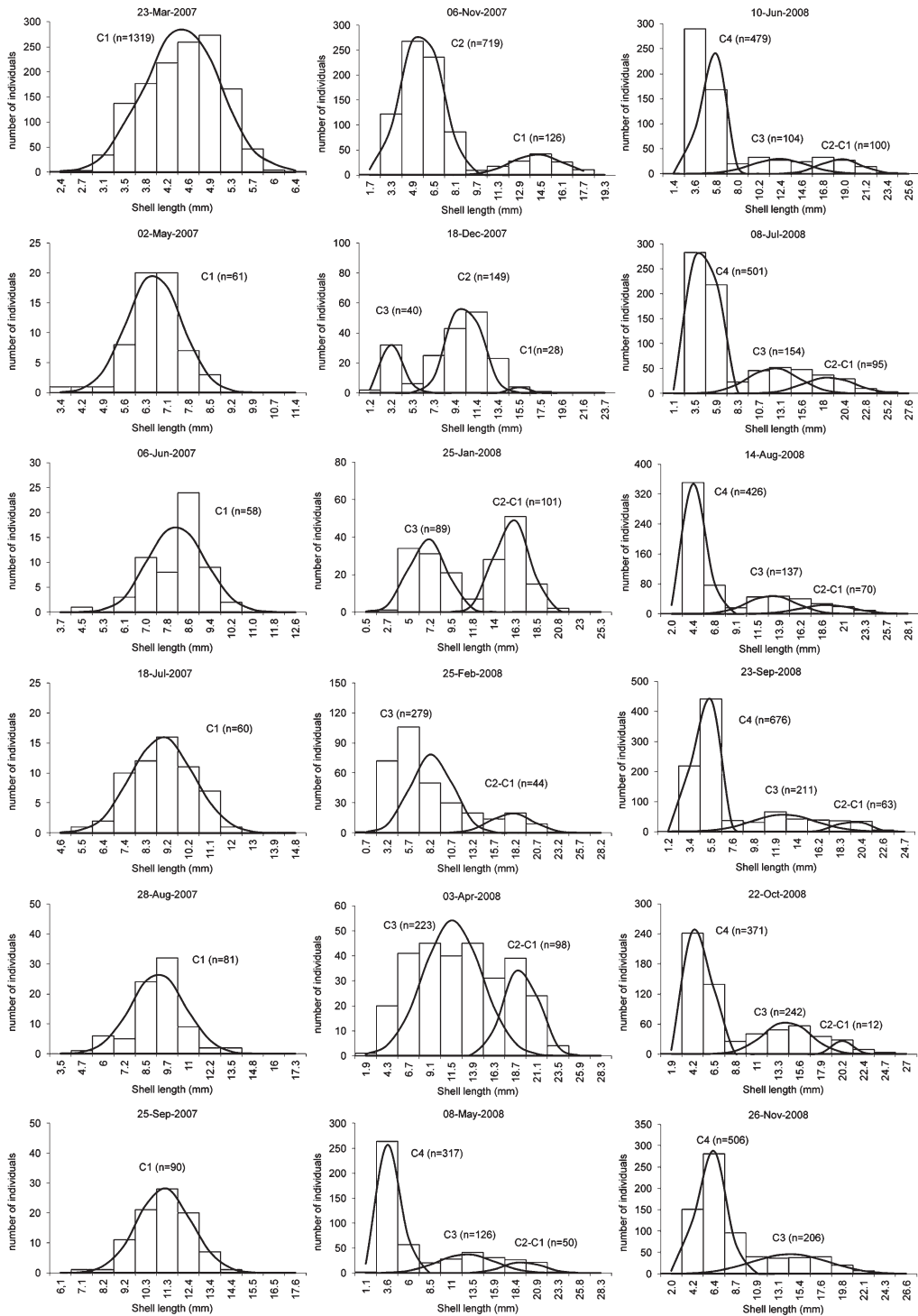
We also measured the height and width of 3,941 individuals from the Coronda and 12,162 from the Santiago Rivers. The measurements on the mussels from the Coronda River had shells of length  $1.35 \pm 0.06$  and  $2.55 \pm 0.02$  times greater than the height and the width, respectively; while the mussels from the Santiago River showed that the shell length was  $1.35 \pm 0.03$  and  $2.27 \pm 0.19$  times greater than the height and the width, respectively. We found that mussels from cages in the Coronda River showed higher length-to-height (L/H:  $t_{(35)} = -1.945$ ,  $P = 0.0297$ ) and length-to-width (L/W:  $t_{(35)} = -1.857$ ,  $P = 0.0358$ ) ratios than those from the Santiago River, but we found no significant differences in height-to-width ratio (H/W:  $t_{(35)} = -0.284$ ,  $P = 0.3890$ ) between the rivers.

#### Individual Growth

Seasonally oscillating VBGF growth model yielded a high degree of goodness of fit for cohort 1, 2, and 3 from the Coronda River, and for cohort 1 to cohort 4 from the Santiago River (Table 1, Fig. 5). In contrast, cohort 4 from the Coronda River and cohort 5 from the Santiago River were excluded from the analysis because they were only constituted by three modal components that prevented us from appropriately fitting the seasonal growth model.

The average growth performance index ( $\phi'$ ) for mussels from the Coronda River ( $3.04 \pm 0.16$  SD, range: 2.93 to 3.23) was higher than Santiago River mussels ( $2.77 \pm 0.18$  SD, range: from 2.57 to 2.99) ( $t_{(5)} = -2.077$ ,  $P = 0.0464$ ). In contrast, the theoretical longevity for mussels sampled from the Santiago River was higher than those from the Coronda River ( $t_{(5)} = 2.080$ ,  $P = 0.0460$ ; Table 1 and 2). We analyzed this spatial difference in growth comparing those cohorts that showed similar values of  $t_0$  (Table 1, Fig. 6). Consistent with the estimations of growth performance index, the *F*-test of the sum of squares between the seasonal growth model fitted to cohort 2 from the Coronda River and cohort 3 from the Santiago River gave significant differences ( $F_{(2,20)} = 28.960$ ,  $P < 0.001$ ) as well as the comparison between cohort 3 from the Coronda River and cohort 4 from the Santiago River ( $F_{(2,16)} = 249.606$ ,  $P < 0.001$ ).

The two-way ANOVA used to analyze temporal variation on growth rates showed no significant interaction between sites and seasons ( $F_{(3,68)} = 1.355$ ,  $P = 0.2639$ ); therefore, we removed the effect of interaction from the final analysis. The ANOVA showed significant differences between sites ( $F_{(1,71)} = 6.536$ ,  $P = 0.0127$ ) and between seasons ( $F_{(3,71)} = 10.084$ ,  $P < 0.0001$ ). In average, growth rate was 1.5 times higher in the Coronda River compared to the Santiago River (Table 2). In both sites, the lowest growth rate occurred during winter. The peak of growth in the Coronda River was in spring and about four times the growth during winter. In the



(continues)



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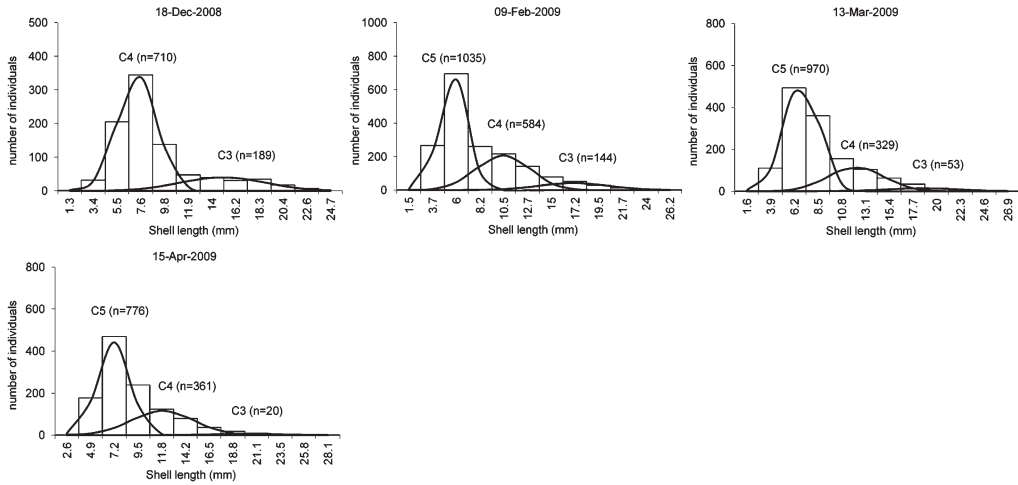


FIG. 3. Length-frequency distribution of *Limnoperna fortunei* from the Santiago River from March 2007 through April 2009; C, cohorts; n, number of mussels from each cohort.

Santiago River, growth peaked during summer and was about six times greater than winter growth (Fig. 7).

Growth-performance indices ( $\phi'$ ) calculated from growth parameters provided in other works that used the von Bertalanffy growth function are summarized in Table 2 along with the shell growth rates reported in different studies carried out in the species' area of origin and other locations where the golden mussel has been introduced. The average growth-performance index of mussels from the Coronda River was significantly higher than the

Bagliardi Beach, Argentina (one-tailed  $t_{(2)} = 3.984, P = 0.0288$ ), it was not different from the Paraná de las Palmas, Argentina (one-tailed  $t_{(2)} = -0.524, P = 0.3262$ ), and it was lower than the index from the Bela Vista Reservoir, Brazil (one-tailed  $t_{(2)} = -5.137, P = 0.0179$ ). In contrast, the average growth-performance index of mussels from the Santiago River was significantly lower than the index from Paraná de las Palmas (one-tailed  $t_{(3)} = -3.666, P = 0.0176$ ) and the Bela Vista Reservoir (one-tailed  $t_{(3)} = -8.667, P = 0.0016$ ) but was not different from the growth-performance index

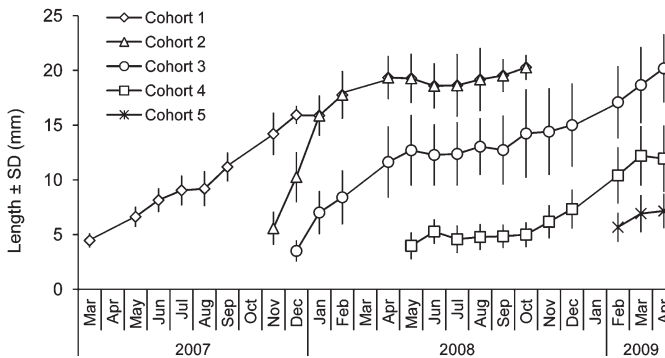


FIG. 4. Cohorts identified from length-frequency distributions. SD, standard deviation.

TABLE 1. Seasonally oscillating von Bertalanffy growth function parameters of cohorts of two populations of *Limnoperna fortunei* from contrasting aquatic environments (the Coronda and Santiago rivers) from the Río de la Plata basin.  $L_{\infty}$  is asymptotic length (mm),  $K$  is the growth constant ( $\text{year}^{-1}$ ),  $t_0$  is age at zero length (fraction of the year),  $C$  is the oscillation amplitude parameter,  $WP$  is the winter point, and  $\phi'$  is the growth performance index. The coefficient of determination for nonlinear regressions estimated according to Brey (2001) is denoted with  $R^2$ , and the number of data points is denoted with  $n$ .

Parameters	Coronda River			Santiago River			
	Cohort 1	Cohort 2	Cohort 3	Cohort 1	Cohort 2	Cohort 3	Cohort 4
$L_{\infty}$	26.55	26.55	26.55	27.55	27.55	27.55	27.55
$K$	2.42	1.22	1.28	0.83	1.31	0.68	0.49
$t_0$	0.18 (Summer)	0.85 (Spring)	1.08 (Summer)	-0.01 (Spring)	0.73 (Winter)	0.81 (Spring)	1.14 (Summer)
$C$	0.610	0.777	1.000	0.552	1.000	1.000	1.000
$WP$	2.50	1.39	1.47	2.40	1.47	0.57	0.98
$\phi'$	3.23	2.93	2.96	2.80	2.99	2.71	2.57
Longevity	1.3	2.6	2.4	3.5	2.3	4.2	6.4
$R^2$	0.997	0.916	0.974	0.981	0.978	0.988	0.979
$n$	8	9	8	17	11	15	11

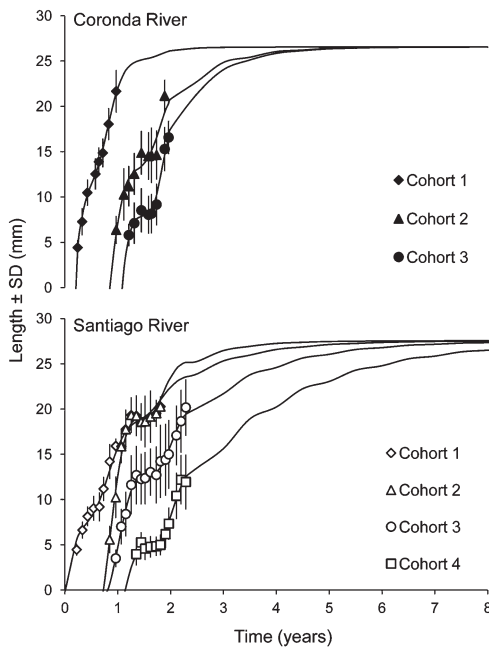


FIG. 5. Seasonally oscillating von Bertalanffy growth curve fitted to each cohort of the two populations of *Limnoperna fortunei* from the Coronda and Santiago rivers. Time is calculated as fractions of the year starting from 1 January 2007.  $SD$ , standard deviation.

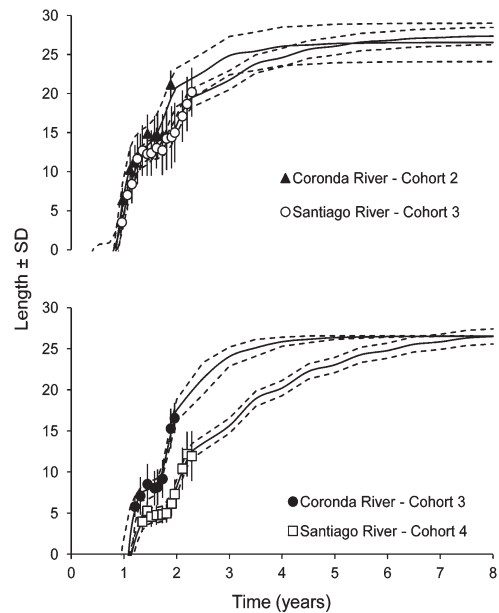


FIG. 6. Seasonally oscillating von Bertalanffy growth curve fitted to cohorts from both populations of *Limnoperna fortunei* with similar  $t_0$  from the Coronda and Santiago rivers. Time is calculated as fractions of the year starting from 1 January 2007. Dotted lines indicate confidence limits of predictive model.

TABLE 2. Growth data (mean  $\pm$  SD) from different populations of *Limnoperna fortunei*. SGVBF, Seasonally oscillating von Bertalanffy growth function; VBGF, Standard von Bertalanffy growth function; SGR, Shell growth rate indicated by the coefficient ( $b$ ) of the linear growth model ( $y = a + bx$ ) or estimated as the difference between the initial and the final shell length divided by the number of days elapsed; ND, No data available. Abbreviations are indicated in Table 1. (\*Study in native distribution range, otherwise from invasive distribution.)

Country	Study Site	Growth Model	SGR (mm day <sup>-1</sup> )	L <sub>∞</sub> (mm)	K (year <sup>-1</sup> )	C	φ'	Longevity	Reference
Argentina	Paraná de las Palmas	SGVBF	ND	35.00	1	1	3.09	3	Boltovskoy & Cataldo (1999)
	Bagliardi Beach	VBGF	ND	36.00	0.35 $\pm$ 0.02	ND	2.66 $\pm$ 0.03	3.5	Maroñas et al. (2003)
	Coronda River	Linear	0.056 $\pm$ 0.012	ND	ND	ND	ND	ND	Bonel et al. (2013)
	Santiago River	Linear	0.031 $\pm$ 0.007	ND	ND	ND	ND	ND	Bonel et al. (2013)
	Coronda River	SGVBF	0.045 $\pm$ 0.036	26.55	1.64 $\pm$ 0.67	0.80 $\pm$ 0.20	3.04 $\pm$ 0.16	2.1 $\pm$ 0.7	This paper
Brazil	Santiago River	SGVBF	0.030 $\pm$ 0.035	27.55	0.83 $\pm$ 0.35	0.89 $\pm$ 0.22	2.77 $\pm$ 0.18	4.1 $\pm$ 1.7	This paper
	Guaíba Lake	ND	0.041 $\pm$ 0.011	ND	ND	ND	ND	ND	dos Santos et al. (2008)
	Bela Vista Reservoir	VBGF	ND	38.00	2.36 $\pm$ 0.41	ND	3.53 $\pm$ 0.07	3.1	Belz et al. (2010)
	Uji River	ND	0.048 $\pm$ 0.007	ND	ND	ND	ND	2	Iwasaki & Uryu (1998)
Japan	Lake Biwa-Yodo River system	ND	0.041	ND	ND	ND	ND	2	Magara et al. (2001)
China*	Lake Ohshio	ND	0.026	ND	ND	ND	ND	ND	Nakano et al. (2011)
	Plover Cove	ND	0.057 $\pm$ 0.010	ND	ND	ND	ND	2	Morton (1977)

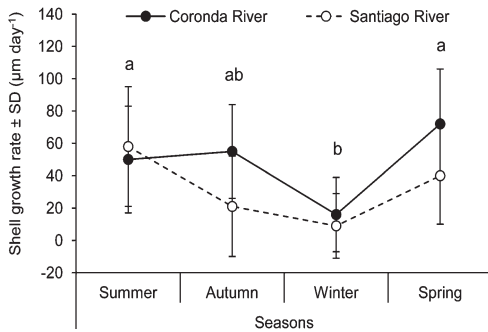


FIG. 7. Results of two-way ANOVA and Tukey HSD test for seasonal variation of growth rate (GR). Different letters (a-b) indicate significant differences of GR between seasons. SD, standard deviation.

from the Bagliardi Beach (one-tailed  $t_{(3)} = 1.222, P = 0.8455$ ).

Body Weight

We found significant differences in the weight-to-length relationship between individuals from both study sites ( $F_{(2,21)} = 3.467, P = 0.0105$ ). The allometric regressions indicated a negative allometry for mussels from the Coronda River ( $b = 1.995$ ; Fig. 8A) and the Santiago River ( $b = 1.686$ ; Fig. 8A). In both allometric models, we observed an increase in the standard deviation of AFDW in mussels with length greater than 13 mm (Fig. 8A). The ANCOVA we used to explain the spatial differences of weight and length showed a significant interaction between length and sites ( $F_{(1,19)} = 8.807, P = 0.0079$ ; Fig. 8B). The significant interaction between site and length means that the slopes of the relationship between length and AFDW has different slopes for the different sites with the AFDW being greater at the Coronda River compared to the Santiago River, as mussels get bigger, but the opposite in small mussels (Fig. 8B).

The two-way ANOVA used to explain seasonal variation of AFDW between sites and seasons showed significant differences between seasons ( $F_{(3,29)} = 5.215, P = 0.0053$ ) but no significant differences between sites ( $F_{(1,29)} = 0.4967, P = 0.4866$ ) nor were there significant interactions of both variables ( $F_{(3,29)} = 0.8408, P = 0.4826$ ). In both sites, the highest weight was observed during spring, and it was about two to six times the weight in the summer

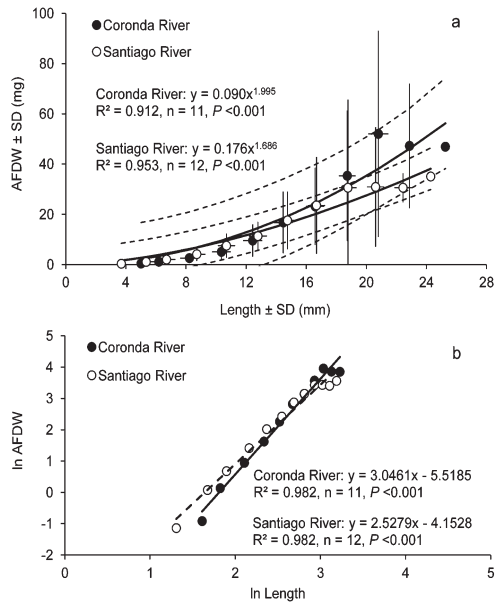


FIG. 8. Allometric relationships. A) Allometric relationships (AFDW =  $a$  Length <sup>$b$</sup> ) between shell length and ash-free-dry-weight (AFDW); B) Allometric relationships (ln AFDW =  $a + b$  ln Length) between ln-transformed parameters. SD, standard deviation. Dotted lines indicate confidence limits of predictive model.

and autumn (Fig. 9). Growth rate of shell and AFDW of each cohort in both sites were uncoupled; these variables showed no significant correlation between them (Fig. 10).

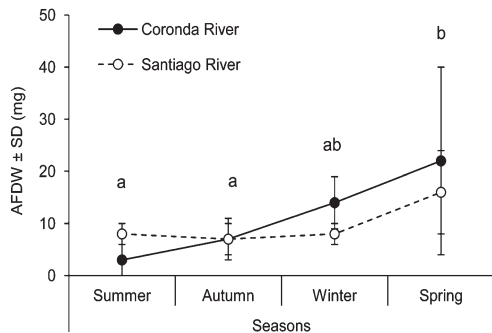


FIG. 9. Results of two-way ANOVA and Tukey HSD test for seasonal variation of ash-free-dry-weight (AFDW). Different letters (a-b) indicate significant differences of AFDW between seasons. SD, standard deviation.

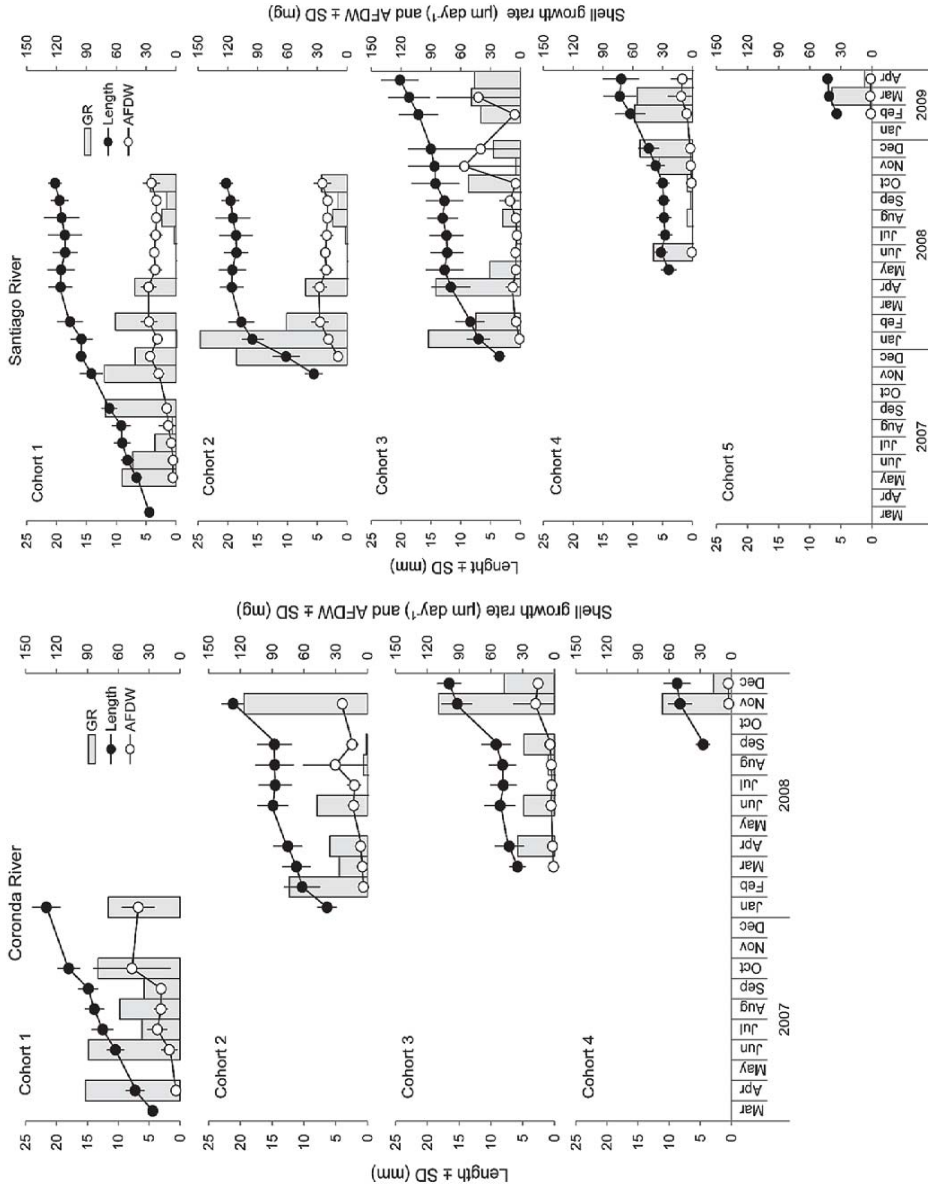


FIG. 10. Growth rate of shell length (GR) and ash-free-dry-weight (AFDW) plotted against shell length (L) increment of each cohort throughout the experimental period at each study site. SD, standard deviation.

## DISCUSSION

The present work has documented for the first time the effects of environmental variability and seasonality on the individual growth and body weight of *Limnoperna fortunei*. We found evidence supporting our predictions that the individual growth and weight of *L. fortunei* were both lower in the more polluted habitat. The comparative approach of this work revealed differences between the growth-performance indices estimated here and those calculated from ecological studies in other ecosystems. We also found the first evidence of temporal variation in shell growth and body weight in both mussel populations. We observed the lowest shell growth rates in winter while the mussel weight became the highest in the spring.

In previous work, Bonel et al. (2013) found that individuals of *L. fortunei* established in highly polluted environments showed a decrease in growth, but the authors indicated that the linearized model was the best one to explain growth on the basis of their data. Therefore, they did not account for the effect of seasonality on growth. In contrast, we found significant evidence of temporal variation in growth, both within and among populations. The average amplitude of the seasonal oscillation for both study sites obtained from the seasonalized von Bertalanffy growth function indicated a high variation in growth between summer and winter (Table 2). This result was consistent with seasonal differences in growth rates, which values were significantly lower during the winter and four to six times higher during the spring and summer in both aquatic environments.

Although we observed similar seasonal patterns in growth between the two populations, we also found spatial differences. When cohorts with similar values of  $t_0$  were compared, we observed that the growth in the Santiago River was significantly lower. In fact, mussels from the more polluted environment showed, on average, lower growth-performance indices than individuals from the less polluted habitat. As opposed to the Coronda River, the Santiago River is subjected to sewage input from large and small coastal cities, industrial waste discharge, and spills of oil and other materials associated with maritime transport and shipyard activities (cf. Bonel et al., 2013). Bivalves exposed to habitats that are heavily polluted

tend to bioaccumulate inorganic and organic contaminants and can respond to stress by exhibiting poor growth and a decline in body weight that can, in turn, negatively affect reproduction (Widdows & Donkin, 1992; Stirling & Okumus, 1994; Alfaro et al., 2008). Our results support and extend the conclusion provided by Bonel et al. (2013) that the high level of pollution in the Santiago River decreased the growth of *L. fortunei*.

Mollusks including bivalves tend to exhibit negative density-dependent growth (Alunno-Bruscia et al., 2000). In these experiments, we did not estimate the density inside the cages and even though we placed a known initial number of mussels inside the cages, densities increased throughout the experiment due to recruitment of larvae produced inside and/or outside the cages. Thus, the negative effect of pollution on the growth of *L. fortunei* might be confounded with negative density-dependent effects. Bonel et al. (2013) found that at high densities these mussels became more elongated – that is, they exhibited higher length-to-width ratios than mussels at the lower densities. Therefore, if density within the cages had become high enough to negatively affect growth, we might have expected to see also an effect on mussel morphology. On the basis of the number of individuals sampled, the abundance of mussels within the cages in the Santiago River appeared to be higher. As indirect evidence of density-dependent effects, we therefore would have expected that the individuals from that site would have been characterized by higher morphometric shell ratios than the mussels from the Coronda. Instead, we found that individuals from the Coronda River showed higher morphometric ratios than those from the Santiago River. According to size-structure, the number of individuals sampled in the Santiago River consisted of a high percentage of small individuals (69% with shell-length =  $5.85 \pm 1.43$  mm), whereas in the Coronda River that size class (shell-length =  $5.92 \pm 1.24$  mm) represented only 28% of the total of individuals collected (Figs. 1 and 3). We thus believe that the density within cages in the Santiago River was not the primary cause affecting mussel growth because the population density pressure caused by small individuals appeared to not be significant to even affect shell morphometrics. Instead, we favor the interpretation that the higher levels of pollution

in the Santiago River was the more influential condition exerting the physiologic stress on the mussels that significantly decreased their growth. Nevertheless, we are aware that our data might be insufficient to support this hypothesis firmly. In order to understand the density-dependent effects upon the biology of *L. fortunei*, future laboratory and field studies are needed.

Previous to the present work no study had compared the growth between different locations where *L. fortunei* had become established. We found that, on average, individuals from the more polluted environment showed a lower growth-performance index than mussels from the less polluted habitat. In addition, the average growth-performance index that we estimated from Boltovskoy & Cataldo (1999) was higher than that from the Santiago River but showed no difference from the one from the Coronda River. In contrast, the index from Maroñas et al. (2003) was lower than the one from the Coronda River and showed no differences in the average value from that of the Santiago River. So far, we have compared growth-performance indices estimated for mussels living in a temperate climate, but the index estimated from a study in a subtropical area (Belz et al., 2010) was significantly higher than the average indices obtained in the present work. The spatial differences in growth-performance indices found in this study emphasize the high variability of growth in response to environmental heterogeneity. Nevertheless, the comparative results between our data and the indices from Maroñas et al. (2003) and Belz et al. (2010) should be regarded with caution. The growth parameters ( $L_{\infty}$  and  $K$ ) in those two studies were estimated by means of the standard von Bertalanffy growth function instead of the seasonalized version used by us and in the work of Boltovskoy & Cataldo (1999). According to Pauly (1990), growth models that do not explicitly consider seasonal oscillations fail to capture an essential aspect of the growth process. We believe that the growth-performance indices that we estimated by means of parameters from these studies may be under- or overestimated, so that the differences we found could be biased.

In this study, we found that the weight-to-length relationships showed a strong negative allometry for mussels from the Coronda and Santiago rivers but that the allometric coefficient was significantly lower for mussels

from the Santiago River. A negative allometric exponent indicates that mussels grew relatively slower in mass than in length (Alunno-Bruscia et al., 2001). In addition, the weight-to-length relationship in mollusks provides a measure of the relative condition or nutritional state of the population (Nalepa et al., 1995). Thus, mussels from a more polluted environment would grow significantly slower in mass versus length than mussels from a less polluted site, reflecting a diminished physiological condition in response to the higher levels of pollution.

The relationship between length and weight and the significant interaction between the covariate (length) and the factor (sites) (Fig. 8B) might be a result of Santiago's mussels allocating more energy for reproduction early on in response to the stressful environmental conditions (Petes et al., 2007) in the Santiago River. Many organisms exhibit early spawning in response to stress (Petes et al., 2007, 2008). In fact, we observed a recruitment event that occurred during the winter period in the Santiago River (cohort 2) but it was absent in the Coronda River. We believe that the physiological stress caused by higher levels of pollution in the Santiago River might have induced those mussels to allocate energy from growth into reproduction and caused that earlier spawning in winter. This hypothesis agrees with the fact that small individuals in the Santiago River showed higher weight than those in the Coronda River, though this relationship changed when individuals became larger. Organisms continually adjust physiological processes and energy allocation in response to varying environmental conditions in order to minimize negative effects on growth, reproduction, and survival (Stoeckmann & Garton, 2001). The hypothesis of energy allocation from growth into reproduction as an adaptive response to stress of the Santiago River's mussels could also explain the difference in growth between the two populations, with the Coronda River's mussels growing faster compared to Santiago River's mussels.

We observed that weight of both populations of *L. fortunei* was significantly higher in spring and lower in summer and autumn. Annual weight variation is commonly the result of cyclical periods of gamete production and release (van Ekrom Schurink & Griffiths, 1993; Steffani & Branch, 2003). As temperatures increase, spawning is initiated and weight loss occurs throughout the summer months until a

minimum weight occurs in late summer to early autumn (Nalepa et al., 1995). Observations on spawning in field populations provide the most reliable evidence of the natural process, but the same conclusions can also be inferred from the appearance of larvae in plankton or the recruitment of juvenile mussels into populations (Seed & Suchanek, 1992). The highest peaks of larval densities and recruitment of *L. fortunei* occurring from late spring through the beginning of autumn in the Coronda and Santiago rivers (Bonel et al., 2013) are consistent with the seasonal changes of body weight observed in this study.

In the present work, we found no correlation between the seasonal patterns of shell and weight growth. In general, shell growth occurs regularly – though at different rates in temperate climates – while weight fluctuates widely with respect to the size at which mussels attain maturity, as a result of cyclical periods of gamete production and release (van Ekrom Schurink & Griffiths, 1993). We observed that weight was highly variable in mussels greater than 13 mm in shell-length (Fig. 10), probably because of the full or partial gamete release that occurred during spring and summer. The highest larval densities reported by Bonel et al. (2013) during those seasons would support our observations. Nevertheless, an apparent decline in weight could also be a result of the rapid change in the covariate, shell length (Hilbish, 1986). According to Palmer (1981), this situation reflects a strategy to increase the habitable volume of shells in anticipation of future soft-tissue growth. Therefore, shell morphology alone may not be a sufficient indicator of the condition of the population (Chase & Bailey, 1999).

In conclusion, our findings demonstrate for the first time how environmental stress and seasonality affect individual growth and body weight of *L. fortunei*, both within and among populations. Different studies concluded that the golden mussel tolerates a wide range of environmental conditions (Ricciardi, 1998; Karatayev et al., 2007; Oliveira et al., 2010), but the lower growth and body weight observed in the mussels from the more polluted sites in this study might be a consequence of diminished physiological condition. Our results confirm and extend the spatial differences in density, shell allometry and growth reported previously by Bonel et al. (2013) and help in our understanding of the influence of environmental

heterogeneity on the biological and ecological processes of *L. fortunei*. In the face of the predicted increase in stressful environmental conditions brought about by climate change, future laboratory and field studies are needed to garner a better comprehension of the adaptive responses of this worldwide invasive species to multiple stressors over space and time.

#### ACKNOWLEDGEMENTS

We thank the anonymous referees for their critical review of the manuscript and constructive suggestions. NB was supported by a CONICET fellowship to carry out this research. NB thanks Dr. G. A. Darrigran (Staff researcher, CONICET) for introducing him to this invasive species. NB is also grateful to Dr. N. J. Cazzaniga (Staff researcher, CIC) for his support and to Dr. C. Ituarte (Staff researcher, CONICET) for providing helpful suggestions on early stages of this manuscript. The authors express their gratitude to S. Teck and D. F. Haggerty for their comments and edits on the manuscript, and to the La Plata Port Consortium Management (La Plata city) and the Amphibious Engineer Battalion (Santo Tomé city, Santa Fe province) for providing access to the restricted area where the study was carried out.

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